Vibrational Spectroscopy 87 (2016) 149–156

Contents lists available at ScienceDirect

Vibrational Spectroscopy

journal homepage: www.elsevier.com/locate/vibspec

Monitoring of sulfated polysaccharide content in marine sponges by Raman spectroscopy

Lenize F. Maia[a,*], Tatiani A. Gonzaga[a], Rafael G. Carvalho[b], Camila M. Leite[b], Gisele Lobo-Hajdu[c], Jair A.K. Aguiar[b], Howell G.M. Edwards[d], Luiz F.C. de Oliveira[a]

[a] NEEM—Núcleo de Espectroscopia e Estrutura Molecular, Departamento de Química, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil
[b] Laboratório de Análise de Glicoconjugados, Dep. Bioquímica, Universidade Federal de Juiz de Fora, MG, Brazil
[c] Laboratório de Genética Marinha, Departamento de Genética, Universidade do Estado do Rio de Janeiro, RJ, Brazil
[d] Chemical and Forensic Sciences, School of Life Sciences, University of Bradford, Bradford, West Yorkshire BD7 1DP, United Kingdom, United Kingdom

ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 3 October 2016
Accepted 3 October 2016
Available online 5 October 2016

Keywords:
Marine sponges
Polymastia janeirensis
Echinodictyum dendroides
Dragmacidon reticulatum
Raman spectroscopy
sulfated polysaccharides

ABSTRACT

In this work sulfated polyssacharides from the marine sponges *Polymastia janeirensis*, *Echinodictyum dendroides* and *Dragmacidon reticulatum* have been analyzed by Raman spectroscopy as well as by biochemical analysis. The results showed that Raman spectroscopy can be applied as a screening method in monitoring the separation of ionic compounds such as sulfated polysaccharides in marine biological systems. The technique has been proven to be suitable in identifying sulfated polysaccharides rather than glycosaminoglycans from sponge tissues.

1. Introduction

Sulfated polysaccharides from marine sponges are chemical compounds that contain specific complex molecules [1–7], identified to be involved in cell-cell recognition, cell adhesion [8], cell development, differentiation and cell-cell interaction [9–11]. Different species of sponges are known to synthesize polysaccharides with a high diversity of sugar moieties and with high sulfate content [3,6,12,13]. Glyconectins are examples of sulfated polysaccharides with a cell adhesion function forming a new class of proteoglycan-like molecules found in several sponges [5,6,12]. The biological activities of sulfated polysaccharides from algae, invertebrates, vertebrates and plants have been well documented [14–18]. Sulfated polysaccharides from marine invertebrates such as echinoderms and tunicates have demonstrated potent anticoagulant activities [19–21] and from sponges, the anti-HIV bioactivity has recently been addressed [13].

In this work, we have investigated the occurrence of sulfated polysaccharides from the endemic species *Polymastia janeirensis, Echinodictyum dendroides* and the species *Dragmacidon reticulatum,* which occur widely in the Eastern and Western Atlantic. It is worth mentioning that aqueous and crude organic extracts from *P. janeirensis* have shown antineoplastic, antichemotactic, antibacterial [22] and antiviral activities [23] and that they were also capable of inducing cell death apoptosis through an oxidative mechanism [24]. The characterization of sulfated polysaccharides here was made by a combination of biochemical methods and Raman spectroscopy, which have been used as an alternative method for identification of the sugar content in crude extracts and fractionated samples isolated during the purification process. Since Raman spectroscopy is now a method of choice for obtaining “molecular fingerprints” in a diversity of biomolecular systems, this may become an interesting option for the analysis of sulfated polysaccharides with the added advantage of being rapid, non-destructive of the specimen and for which no chemical or mechanical pre-treatment is necessary. This work is the first report on the characterization of polysaccharides isolated from marine invertebrates by Raman spectroscopy.

* Corresponding author.
E-mail address: lenmaia@uol.com.br (L.F. Maia).

http://dx.doi.org/10.1016/j.vibspec.2016.10.002
0924-2031/

2. Experimental

2.1. Sponge collection

Samples of *Polymastia janeirensis, Dragmacidon reticulatum* and *Echinodictyum dendroides* were collected in December 2014 by scuba divers at Angra dos Reis in the coastal region near Rio de Janeiro (23°02′20″S/23°03′25″O). After collection, the sponges were immediately frozen in dry ice.

2.2. Preparation and fractionation of crude extracts

2.2.1. Aqueous extraction

Aqueous extracts were prepared as described by da Silva et al. [23]. Sponge materials were crushed with sand and water three times for 30 min, and the extract was subsequently filtered and freeze-dried.

2.2.2. Extraction of polysaccharides by proteolytic digestion

Polysaccharides were extracted from wet tissues of each sponge species by proteolytic digestion using ALCALASE® (Millipore) (Tris-HCl 0.5 mol·L^{-1}, pH 9.0) at 60 °C for 24 h. Precipitation of proteins was achieved by adding trichloroacetic acid (TCA 10%) to the supernatant liquid in the presence of NaCl 1.0 mol·L^{-1}, with the proteins being removed by centrifugation (3000 rpm, 15 min). To the supernatant solution ethanol has been added under agitation, in a quantity of 2.5 times the total volume of the solution, which was then left to stand overnight at −20 °C. The precipitate formed was collected by centrifugation and dried under vacuum.

2.2.3. Fractionation of the sulfated polysaccharides by anion exchange chromatography on Q-Sepharose FF

Crude extracts of the sulfated polysaccharides from the marine sponges (∼25 mg from each species) were added to a Q-Sepharose column (5 mL) equilibrated with water. Crude extract fractionation was performed by elution with solutions of increasing NaCl concentration: namely, 0 (zero), 0.5, 1.0 and 2.0 mol·L^{-1}, termed F1–F4.

2.2.4. Agarose gel electrophoresis

Crude extracts and their fractions obtained from anion exchange chromatography were submitted to agarose gel electrophoresis as previously described in the literature [25]. Briefly, 5–50 μg of each sample was applied to agarose gel slabs in a 0.05 mol·L^{-1} 1,3-diaminopropane-acetate buffer at pH 9 (PDA). After fixation with cetyltrimethylammonium bromide (CETAVLON) and Toluidine Blue staining solution, the sulfated polysaccharides were quantified by densitometry of the gel slabs (Epson Expression 1680 Flatbed Scanner, with QuickScan Win 2000, Helena Laboratories, Beaumont, TX, USA).

2.3. Physico-chemical characterization

2.3.1. Chemical analysis

Aminosugars were determined after acid hydrolysis (4 mol·L^{-1} HCl for 6 h at 100 °C) by a modified Elson–Morgan reaction [26]. Uronic acid was determined by a modification of the carbazole reaction [27]. Total inorganic sulfate was measured by the barium chloride-gelatin method after acid hydrolysis (8 mol·L^{-1} HCl for 6 h at 100 °C), as previously described [28], and the soluble protein was measured by a modified Lowry method with bicinchoninic acid (BCA Protein Kit Assay, Pierce, IL, USA), using bovine serum albumin as a standard [29]. Total sugars were measured by the phenol sulfuric method [30]. Standard samples of known concentrations were used to build a standard curve for comparison.

2.3.2. Enzymatic Degradation

Enzymatic treatment of the sponge crude extracts with chondroitinase AC from *Flavobacterium heparinum* was performed as described by [31], but this was not possible to degrade any glycans under conditions that completely digest the respective enzyme substrates.

2.3.3. Polyacrylamide Gel Electrophoresis

The molecular mass of the sulfated polysaccharides extracted from sponge was determined by polyacrylamide gel electrophoresis as previously described [32] using dextran sulfate (8 kDa), chondroitin 4-sulfate (26 kDa) and chondroitin 6-sulfate (67 kDa) as molecular mass standards [33]. Estimation of the molecular masses of acidic mucopolysaccharides was done by polyacrylamide gel electrophoresis.

2.3.4. Raman spectroscopy

Fourier-transform Raman spectra were recorded using a Bruker RFS 100 spectrometer and an Nd:YAG laser operating at 1064 nm, equipped with a Ge detector cooled with liquid nitrogen. A spectral resolution of 4 cm^{-1} was used, and good signal-to-noise ratios were obtained with 500 and 1000 interferogram scans accumulated, using a range of laser powers at the sample between 100 and130 mW. Crude extracts and fractionated samples were obtained as a solid material, on which two series of independent Raman measurements were carried out and for each set a different laser power was applied. The representative spectra were measured with 1000 scans and a laser power of 130 mW.

3. Results and discussion

We investigated the occurrence of polysaccharides from three species of marine sponges: *Polymastia janeirensis, Echinodictyum dendroides* and *Dragmacidon reticulatum*. Crude extracts and fractions obtained after fractionation were characterized by biochemical analysis and Raman spectroscopy. The crude extracts from each sponge exhibited a distinct mobility during agarose gel electrophoresis analysis (Fig. S1 and S2). However, the Raman spectroscopic analysis showed similar spectra with bands attributed to proteins and carbohydrates (Fig. 1). The most characteristic vibrational modes of the CONH group from proteins were observed at *ca*. 1666 (amide I), 1247 cm^{-1} (amide III) [34], and modes related to CH and COH groups from carbohydrates were detected at *ca*. 1353 cm^{-1} δ(COH), 1274 cm^{-1} δ(CH_2OH,COH), 1081 cm^{-1} δ(C_1H, COH), 1059 cm^{-1} δ(CO, COH), 844 cm^{-1} δ(CH anomeric) and 434 cm^{-1}δ(skeleton) [35–38]. It is important to notice that in Fig. 1 as well as in the other figures with Raman spectra, components of a rotation-vibration combination band show up with the Q-branch observed in the Raman spectrum at *ca*. 590 cm^{-1}, due to strong water vapour absorption [39].

Each species showed sulfated polysaccharides obtained from anion exchange chromatography with different migration patterns (F1–F4) by agarose gel electrophoresis analysis (Fig. S3). Polyacrylamide gel electrophoresis (PAGE) showed sulfated polysaccharides with modal molecular masses ranging from 8 to more than 67 kDa (Table 1). Hexosamine, inorganic sulfate, and uronic acid determinations were performed to ascertain the nature of polysaccharide present in the sponge tissues (Table S1). The main goal was to present Raman spectroscopy as a screening method in monitoring the sugar composition of crude extracts and fractioned samples. The characterization of sulfated polysaccharides has been made based on the characteristic “fingerprint” Raman bands attributed to sulfate groups, glycosidic linkages, the COH and the *N*-acetyl groups [40–44].